Understanding Cells in a New Way with Three-Dimensional Models

THE recent completion of the Department of Energy's Human Genome Project indicates the remarkable progress scientists have made in understanding the genetic basis of life. The Genomes to Life Program, the successor to the Human Genome Project, has an even more ambitious goal: to understand life in a comprehensive and integrated manner. The initiative is an example of systems biology, the study of tissues as integrated systems rather than as isolated parts, such as individual genes, proteins, or chemical reactions.

"Decades of experiments on isolated chemical pathways or proteins laid the foundation for the study of complex biological systems. Now, the increasing interest in systems biology reflects the fact that we do not yet have a comprehensive understanding of how a cell functions," says Livermore physicist Andrew Quong. He says that progress in systems biology requires a multidisciplinary effort involving experts in biology, biochemistry, molecular biology, chemistry, physics, and computer science. "Only when experts from various disciplines work together on a computational model can we begin to understand the interactions between genes and the thousands of subcellular components and chemical reactions, many of them linked in subtle ways to one another," says Quong.

Ultimately, advanced computational models—based on sound chemical and physical principles and new laboratory experiments—running on the fastest supercomputers will permit scientists to visualize and understand the complex interactions and changes within a simulated cell. For example, a scientist will one day be able to predict a cell's response to different mixes of nutrients or exposure to a drug or a toxin from a pathogen.

Quong notes that widely used, but relatively simple circuit diagrams treat cellular processes as dimensionless electrical circuits. Previous attempts at computational models have treated only a small set of chemical reactions. By contrast, Livermore scientists use models that combine many more reactions, and, where possible, they extend the simulations to realistically portray a cell in three dimensions.

Quong's project is to develop a three-dimensional (3D) model of calcium ion transport within epithelial cells, which line all body cavities, including the lungs, digestive track, and kidneys. The research is funded by the Laboratory Directed Research and Development program. Calcium ions are fundamental signaling ions in cells and play a role in many cellular processes, from cell division to muscle contraction. Quong's research is among the first attempts at modeling cellular processes to take advantage of massively parallel supercomputers, which use many microprocessors working in tandem.

The research builds on Livermore's experience in computational biology, supercomputing, biosecurity, and multiscale modeling of materials and chemical reactions. The effort also involves laboratory experiments done by Livermore biologists to supply essential data for the developing model.

The immediate payoff from the research will be increased understanding of a key cellular function, but the Livermore team hopes that the model will lead to new insights into the interactions between pathogens (viruses or bacteria) and human host cells. These insights will likely result in advances to control and prevent disease as well as initiatives in homeland security to detect and thwart any attempt at biological terrorism.

Learning from High Explosives

Quong's team is modeling the flow of calcium ions inside kidney epithelial cells using ALE3D, a computer code originally developed at Lawrence Livermore for studying the detonation of high explosives. ALE3D is part of the family of codes belonging to the Advanced Simulation and Computing program, an element of the National Nuclear Security Administration's Stockpile Stewardship Program to ensure the safety and reliability of the nation's nuclear deterrent.

A code for high explosives might not seem applicable for mimicking cellular processes. However, the code's flexibility allows it to model chemical reactions and ions within a cell much as it tracks chemical reactions and the transport of molecules and ions created by chemical explosives.

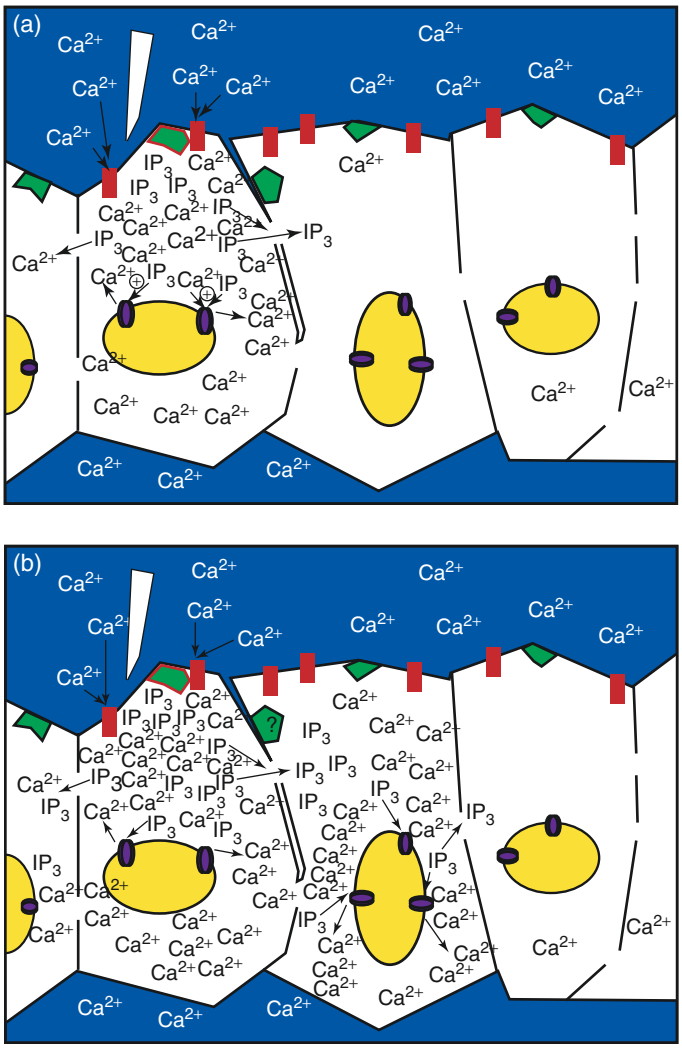
Team member and chemist Albert Nichols notes that the chemistry and underlying physics in both a functioning cell and a high-explosives detonation are the same. Some adjustments must be made, however, for enzymes, which are not found in inorganic systems. Nichols also points out that in models of

purely chemical systems, reactions move toward thermodynamic equilibrium, whereas in a cell, equilibrium means death.

For the cell study, ALE3D tracks waves of calcium ions within a 3D mesh that corresponds to the volume of several adjoining epithelial cells. "Our goal is to track the flow of calcium ions at any point in space inside the cell," says Quong.

Epithelial cells are barriers that protect the body from the external world and inhibit and control the movement of water, molecules, and ions across these barriers. Quong notes that a pathogen's first interaction with a human is with epithelial cells, so understanding the signaling and ion transport pathways in these cells has important applications to human health and protecting against bioterrorist attacks.

From among the large family of different epithelial cells, the team decided on those found in the kidney. These cells can be grown easily in culture. Also, they have a roughly cubic shape, with one highly specialized side facing the outside environment. This asymmetric design can be represented realistically with ALE3D.



Waves of calcium ions are believed to be an important signaling mechanism as they move within and across epithelial cells. The waves are coordinated by inositol-1,4,5-triphosphate (IP_3), which binds to certain cell receptors. The diagram illustrates the flow of calcium and IP_3 molecules from (a) one cell to (b) its neighbors.

Focus on Calcium Waves

In simulating the transport of calcium ions in kidney epithelial cells, the model includes the movement of inositol-1,4,5-triphosphate (called IP_3) molecules because they coordinate the release of calcium ions in waves. The waves, lasting several seconds each, are believed to be an important signaling mechanism as they move within and across epithelial cells. By binding to receptors, which are folded proteins located within the cell, IP_3 molecules trigger the release of calcium.

Although there have been other models of calcium waves, they have been limited to one or two dimensions. Says Quong, "We're constructing for the first time a 3D model that is consistent with experimental data and is based upon valid physiology and chemistry."

One goal of the Livermore model is to accurately reflect where calcium is stored within the cell prior to being released as part of a wave. Calcium ions are often found sequestered in certain organelles, specialized cell parts analogous to organs. A series of laboratory experiments on kidney epithelial cells, grown by Livermore molecular biologist Michael Thelen, will reveal the location of calcium-containing organelles.

The experiments will use antibodies labeled with fluorescent dyes to measure and visualize the calcium. The data will then be fed into the model.

Experiments Underpin Model

Thelen is also planning to image live cells with a confocal microscope. This type of microscope focuses light in a narrow plane, thereby allowing images of progressively deeper slices through a live cell. The observations will be compared with those gathered from secondary ion mass spectrometry (SIMS) imaging. (See the box on p. 17.)

Although the model is still incomplete, Quong has successfully used it to perform simulations based on published data from laboratory studies of kidney epithelial cells. The simulations model a group of several cells measuring about 25 micrometers in diameter. They show the initiation of calcium waves within a single cell and the propagation of the waves through neighboring cells. The simulations, with a resolution of about 1 micrometer, were completed on several Livermore supercomputers processing in parallel.

Visualizing Chemistry

Systems biology demands innovative experiments and instrumentation. Although not new to chemists, secondary ion mass spectrometry (SIMS) is beginning to be applied by biologists. With its extreme sensitivity (a few parts per billion) and spatial resolution (50 to 100 nanometers), SIMS is a powerful technique.

SIMS uses a stream of energetic ions that bombard the surface of a material under investigation. Upon impact, these ions generate positively and negatively charged ions, which are gathered by electrically charged lenses, imaged, and identified.

Secondary ion mass spectrometers are typically used in industry to characterize materials and to examine the surface of semiconductors and polymers. Livermore's three machines are used to map the surfaces of mirrors and optics for the Department of Energy's National Ignition Facility, now under construction at Livermore.

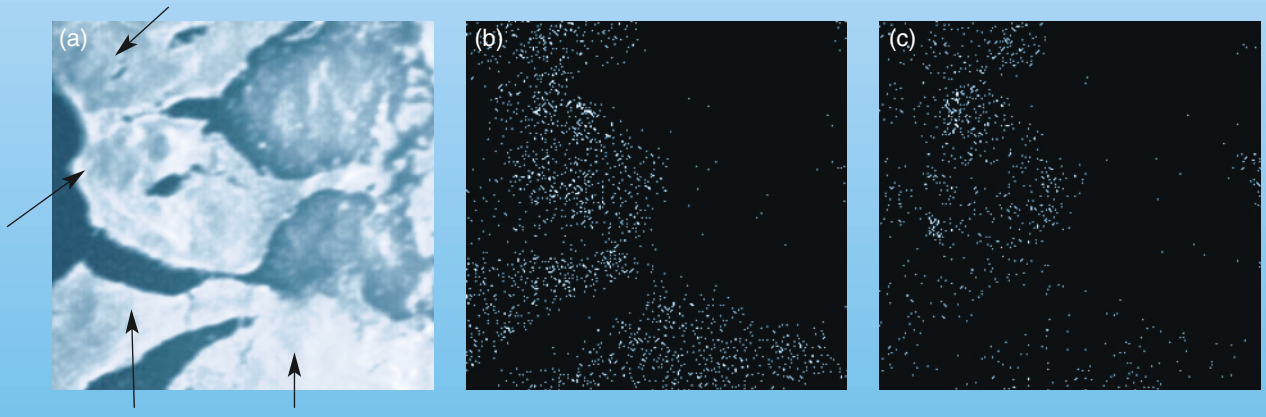
SIMS is unique because it can yield a map of any selected molecules or ions of interest. This is an important feature for biological applications because the alternative in many cases requires homogenizing many cells and then testing for the presence of the molecules or ions. The result is an average of a cell population, with no information about location within a cell.

"Biological processes are dependent upon spatial localization," says Livermore biochemist Judy Quong. Quong is the principal investigator of a Laboratory Directed Research and Development project studying the application of SIMS to subcellular imaging. One area of research seeks to determine the distribution of PhIP (2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine) in cells. One of the mutagenic compounds known as heterocyclic amines, PhIP is a carcinogen that has been consistently demonstrated to cause dose-dependent mammary and prostate tumors in rats. Another area of research is using SIMS to determine the spatial resolution of isotopically labeled cancer drugs in cells. Improved understanding of the distribution of PhIP and cancer drugs in cells could lead to more effective cancer treatment.

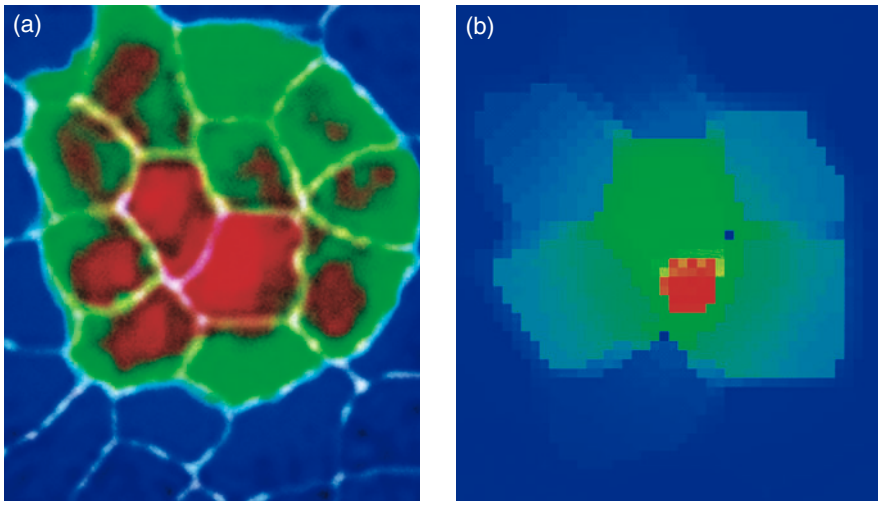
Working with physicists Ian Hutcheon and Kuang Jen Wu, Quong uses two different mass spectrometers, a recently acquired static time-of-flight secondary ion mass spectrometer and a dynamic secondary ion mass spectrometer. The Laboratory will be acquiring a new nanoscale dynamic secondary ion mass spectrometer (NanoSIMS), which has a spatial resolution of 50 to 100 nanometers and greater sensitivity than Livermore's current dynamic instrument. NanoSIMS will be only the second such machine in the nation dedicated to biological research.

Because SIMS can detect any element, it is an ideal technique for helping physicist Andrew Quong's team locate calcium that is sequestered in cellular organelles, specialized cell parts analogous to an organ. SIMS will be able to generate a horizontal and vertical profile of the calcium distribution within frozen cells. The results will be compared to those gained from standard laboratory methods that use radioisotopes in live cells to locate calcium.

Biochemist Quong says she is hopeful that more Livermore biologists will take advantage of SIMS's capabilities. "We're trying to introduce biologists to new techniques that are normally used only by chemists and materials scientists."



To locate the three-dimensional position of calcium ions before they are released in a wave, the modeling team will be using secondary ion mass spectrometry (SIMS). Unlike electron microscopy, SIMS can detect any ion or molecule of choice, as seen in these images. (a) A scanning electron microscope image of clusters of human epithelial cells indicated by the arrows. Although the image shows the morphology of the cells, it cannot reveal the presence of selected molecules. (b) An image of the same cells taken by a secondary ion mass spectrometer shows the location of phosphocholine, a molecule found in cell membranes. (c) A SIMS image of the same cells shows that the PhIP mutagen is also present in this cell membrane.



A Livermore code simulating the flow of calcium ions compares favorably to experimental images of calcium ion waves. (a) An image from confocal microscopy taken by Japanese scientists captures a wave of calcium ions (bound to a red dye) that began in one epithelial cell and spread to its neighbors. (Image reprinted with permission from *Science* **284**, May 28, 1999, 1529. Copyrighted 1999, American Association for the Advancement of Science.) (b) The Livermore code simulates a wave of calcium ions (in red) triggered by the cell located in the center. The simulated cells measure about 25 micrometers across. The white lines represent cell membranes and the missing portions indicate gaps in the membrane where calcium ions pass from one cell to another.

Understanding the flow of ions in epithelial cells may help scientists treat kidney and lung diseases, says Quong. For example, calcium concentration affects the movement of cilia (tiny brushes) that are found in lung epithelial cells. The cilia catch and move pathogens and foreign particles that are trapped in mucus secreted by the cells. However, people suffering from cystic fibrosis, the most common genetic defect among Caucasians, produce abnormally thick mucus that can make breathing difficult and inhibit the movement and effectiveness of the cilia. The thick mucus is believed to be caused by the faulty transport of sodium and chloride ions in and out of the cells. This variable of ion transport will be incorporated into the model.

Biology's New Approach

The Livermore model is pointing the way toward a new approach to understanding how cells operate and diseases begin and progress. The eventual result will likely make the biological sciences more predictive. "A cell has so many interacting pathways that we must understand how everything is related, and computational models can help us do that," says Quong.

Advanced models of chemical pathways in the cell are important to both human health and national security. Indeed, the research directly supports Livermore's national security mission by helping scientists study issues related to biosecurity, such as the effects of pathogens on cell function and host–pathogen interactions.

"Our long-range goal is to develop tools for homeland security and health care," Quong says. Understanding the interactions between a host cell and a pathogen will help scientists learn how to shut off bacterial toxin production, defeat genetically engineered organisms, defend against new antibiotic-resistant microbes, predict a host cell's response to infections, defend agriculture against pathogens, and develop new drug delivery systems.

Computational models, once reserved for phenomena such as explosions and material fracture but now used for cells and their processes, are sure to become an important tool for scientists, health professionals, and those guarding our national security.

—Arnie Heller

Acknowledgments: The 3D epithelial cell modeling team consists of Michael Colvin, Aaron Golumbfskie, Kenneth Kim, Alison Kubota, Christopher Mundy, Albert Nichols, Andrew Quong, Judy Quong, and Michael Thelen.

Key Words: Advanced Simulation and Computing program, ALE3D code, biosecurity, calcium ion waves, confocal microscope, cystic fibrosis, Genomes to Life Program, homeland security, Human Genome Project, secondary ion mass spectrometry (SIMS), systems biology.

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